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Microbiological, physicochemical and sensory assessment of improved Kunun-Zaki beverage made from Millet and stored under different storage conditions

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ABSTRACT
The Production, Microbial,Physicochemical and Sensory quaiity Assessment of Kunun-zaki from millet using improved and traditional method as control was undertaken and stored under two different storage conditions (i.e. ambient and refrigerated temperatures). Microbial Analysis of the improved product sample stored at both conditions gave a Total Viable Count value ranged between 0.1 x 10^4 - 1.5 x 10^4 cfu/ml, Yeast count of 0.2 x 10^4 - 0.8 x 10^4cfu/ml with no growth of Coliform, *Staphylococcus* and *Salmonella* was observed while traditional process sample gave a higher value of Total Viable Count of 1.0 x 10^4 - 2.0 x 10^4 cfu/ml, 1.0 x 10^4 - 1.5 x 10^4 cfu/ml Coliform count, 0.2 x 10^4 - 2.0 x 10^4 .cfu/ml of Yeast count. The Microbes recovered were *Micrococcus acidophilus, Streptococcus lactis, Pseudomonas aerogenosa, Lactobacillus plantarium, Bacillus subtilis, Escherichia coli, Rhizopus nigrican, Penicillium sp* and *Saccharomyces cerevisiae* The pH value for the two samples ranged between 3.0 – 4.6, Total soluble ranged between 5.6 - 6.8% and Titratable Acidity were moderate. Sensory Evaluation on Overall acceptability shows slight significant difference p (<0.05). Overall assessment indicates that the improved product refrigerated possesses better taste, low viable count, no pathogenic organism and of high storage stability.

Keywords: fermented, beverage, millet, yeast count, kunun, stability

INTRODUCTION

Kunun-zaki is an indigenous fermented non-alcoholic beverage that is widely consumed for its thirst quenching properties though can be consumed throughout the year and it is extensively consumed during dry season. The beverage is produced from fermented millet, sorghum, guinea corn and maize in decrease order of preference [1]. Kunun produced from millet is more preferable to that made from other cereals due to its high nutritional requirement. It can be taken as refreshment and for sedative (laxative) purpose when served chilled [2].

The nutritional components of kunun- zaki is 85% - 89% moisture content, 9.84 – 12% carbohydrate, 1.56 – 3% protein, 0.01- 30% fats and 0.16 – 0.75% ash [3].

Its crude method of production and improper sanitary packaging condition predisposes kunun-zaki to microbial contamination [4]. Research has revealed that the micro-biological quality of pipe-borne water and well water supply to some communities in Nigeria is poor with coliform count exceeding the level recommended by WHO [5]. Its local method of production and sales exposes the beverage to a high level of contaminant by pathogenic organism through various means and these pathogens may cause diseases (i.e. typhoid by salmonella) and may reached their hazardous level [6].

The preparation of this beverage has become technology in many homes in the rural communities and more recently in the urban areas where more women have developed the skill of production and commercial production has helped to alleviate poverty among the people in Nigeria. Many women have been able to set-up small scale commercial production of kunun due to support from the government through poverty alleviation scheme [7].

This research work is carried out with the aim of producing kunun under hygienic condition, improving on the traditional production process with the hope of maintaining the microbiological quality of the product i.e. less microbial count and storage stability.

MATERIALS AND METHODS

Materials: Material content for this beverage are 2kg of millet purchased at Ilaro market, 3.8kg of ginger, 4kg of cloves and substantial quantity of sugar was added to taste.

***Production of Kunun-Zaki*:** The Beverage was produced using two processing methods. The improved method is illustrated in the flow diagram in Fig. 1 below while the traditional process involves the steeping of millet grain in water for 24hrs, wet mill with spices (ginger, cloves and pepper). Wet sieving, Decanting and partial gelatinization of the slurry by dividing it into two unequal portions. 75% portion boiled with addition of calculated amount of water. The slurry cooled to 60 – 65^{o}C and the remaining 25% were added & mixed thoroughly, sugar was then added and bottled [8].

Isolation of Microbes Associated with Kunun-Zaki
Using Pour Plate Technique: Ten fold dilutions of each kunun samples were made using peptone water. Appropriate dilutions were made and 1ml of each dilution were pour plated aseptically in triplicate plate using Nutrient Agar (NA) for total variable count, MacConkey Agar (MCA) for coliform test, Manitol Salt Agar (MSA) for Staphylococcus count, Bismuth sulfite Agar for Salmonella count, Sabouraud

Dextrose Agar, with Chloramphenicol was used for fungi and yeast. All plates were incubated for 48 hours at 37^{o}C except for fungi count that were incubated at 28±^{o}C for 5days. Colonies were counted on a Gallenkemp colony counter.
Pure culture of each isolates was obtained by streaking the specific colonies on suitable media and incubated appropriately [9].

Identification of Microbial Isolates: Isolation and Identification of bacteria in Kunun zaki samples were done using methods described by [9] and [10]. The fungi and yeast were identified using methods of [11].

Determination of Physicochemical analysis (P^H, Titratable Acidity and Total Soluble): The P^H of Total Soluble was determined using the method described by [12] using the P^H meter. Total Titratable Acidity was determined using [13].
***Sensory Evaluation*:** The samples produced were analyzed using Analysis of Variance. The samples were rated for taste, colour, consistency and general acceptability. Using SPSS statistical version 17 for analysis means were separated using Duncan new multiple range.

RESULT
The results of the Microbial load of the kunun-zaki for improved and traditional process are presented in Table .1 below. The microbial assessment of the mean total viable count of improved method ranges 0.1 x 10^4 - 1.5 x 10^4cfu/ml, yeast count of 0.2 x 10^4 - 0.8 x 10^4cfu/ml with no growth of coliform, Staphylococcus and Salmonella count. This may be attributed to the portable water used. Sample B(Traditional Processing) has a higher Mean Total viable count ranging from 1.0 x 10^4 - 2.0 x 10^4cfu/ml, 1.0 x 10^4 - 1.5 x 10^4cfu/ml coliform, 0.1 x 10^4 - 0.2 x 10^4cfu/ml Salmonella and 0.2 x 10^4 - 2.0 x 10^4cfu/ml of Yeast count. Table 2 shows the Microbes associated with the kunun-zaki beverage. Four different microbes were isolated from the improved method while the traditional method process samples harboured nine microbes. These microbes include *Micrococcus acidophilus, Streptococcus lactis, Pseudomonas aerogenosa, Lactobacillus plantarium, Bacillus subtilis, Escherichia coli Rhizopus nigrican, Penicillium spp,* and *Saccharomyces cerevisiae.* The P^H, Titratable Acidity and Total soluble are presented in Figure 2. The P^H value of kunu-zaki during storage period decreases from 4.6 – 3.0, Titratable Acidity decreases slightly from 0.77ml – 0.70ml ambient. Total soluble ranges from 5.83% - 3.5%(Ambient), 5.50% - 5.68%(Refrigerated). Sensory Evaluation of fresh sample on colour, taste, consistency and overall acceptability shows that there was slight significant difference. Meanwhile improved Refrigerated sample was more acceptable.

DISCUSSION
Being a thirst quenching beverage, kunun-zaki has high moisture content. The proportion of water varies 55 – 98%, the remainder being mostly addictive [15]. All the samples were acidic in nature with P^H 3.0 – 4.6%. It was observed that the pH, titratable acidity and total soluble decreases with increase in the days of storage for the two method . While only the total soluble increases slightly at the refrigerated temperature. It was also observe that the higher the temperature of storage, the more pronounce the decrease in Ph. This is because the higher the temperature, the higher the rate of metabolism of sugar. And hence the higher the rate of acid production by the relevant micro-organisms [19]. This level of acidity has been described by several researchers including [16], [17] and [18] who attributed these to the presence of certain species of lactic acid bacteria, namely *Lactobacillus leichmanni and L fermentum,* during the fermentation process, the low P^H value may have encouraged the growth of yeast seen which predominate the samples. Four microbes were found to be associated with the improved while nine microbes were isolated from the traditional process samples which were *Bacillus subtilis Micrococcus acidophilus, Lactobacillus planetarium, Streptococcus lactis, Escherichia coli, Pseudomonas aerogenosa, Rhizopus nigrican, Penicillium sp* and *Saccharomyces cerevisiae. This agree with the work of* [19]. The presence of some of these organisms are not surprising as most of them are known to thrive in medium rich in fermentable substrates such as sugar which often led to the production of acids after fermentation [20]. [21] reported that L – plantarium was the predominant organism at fermentation responsible for the lactic acid production. *Pseudomonas* sp and Bacillus subtilis found in the traditional sample might be responsible for the changes in taste which normally occurs if not consumed within few hours [22]. The presence of coliform found in the traditional sample was as a result of tap water used which indicates faecal contamination of water and could cause gastroenteritis. The presence of fungi in the traditional sample may be attributed to the nature of the sample since it was observe that yeast and mold are capable of utilizing organic acid [19] and are common spoilage of Carbohydrate food and storage microflora of many cereals [20]. The presence of *Saccharomyces cerevisiae* in both samples is probably they play the same role in flavor development and ability to ferment lactose. This agrees with the earlier work of [18] and [19].

The physico-chemical shows the pH value of the product were acidic in nature and decrease under storage condition [23] which may encourage fungi growth and a low titratable acidity.

Sensory evaluation of the Kunun-zaki shows that on the First day of production, both the improved and traditional processes stored under Refrigerated were wholesome while samples stored under ambient temperature developed a change in taste because the product became soured and this was earlier observed in traditional method by the second day. Storage above 8 days of improved process under refrigeration temperature shows that kunun-zaki still maintains its colour, odour and taste.

The Statistical Analysis shows that there were significant difference in the color . While sample B_1 and B_2 shows no significant difference. p (<0.05).

There were significant difference in the taste and consistency. While sample A_3, B_1 and B_3 shows no significant difference. p (<0.05), due to the storage under ambient temperature.

The overall acceptability shows no significance difference. P (< 0.05), of the improved method. While the traditional method shows slight significance difference.

CONCLUSION

Apparently, the process employed in the production of Kunun-Zaki would affect production quality and acceptability. The study reveals that kunun-zaki beverage produced maintains its colour, odour, taste provided there was constant electricity. The sensory evaluation test for preference multiple comparison test shows there was significance difference on overall acceptability at 5% confidence level with improved method stored under refrigerated temperature with constant supply of electricity has fewer microbial count which are not hazardous to human health and devoid of pathogenic microbes meanwhile the storage of kunun can be improved upon by reducing the initial microbial load through control fermentation of steeped grain use of treated or portable water for preparing the slurry and addition of chemical preservative.

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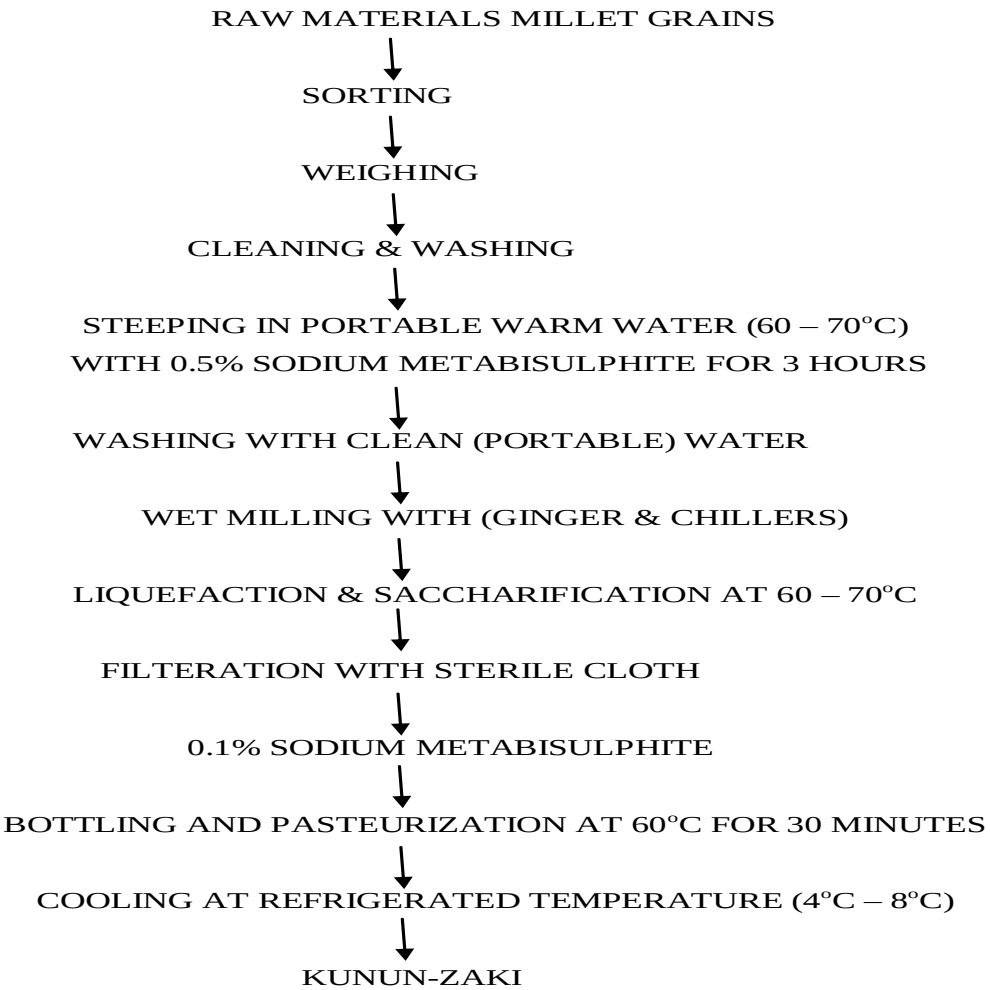

Fig. 1. Flow Chart for Improved Production Process of Kunun- Zaki Beverage [14]

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Table 1. Microbial Count of Kunun – Zaki Beverage of Improved and Traditional Method.

Sample Code	Total Viable count (Cfu/ml)	Total coliform count (Cfu/ml)	S. aureus Count Cfu/ml)	Samonella count (Cfu/ml)	Fungi count (Cfu/ml)	Yeast count (Cfu/ml)
A_1	0.1×10^4	-	-	-	-	0.2×10^4
A_2	1.0×10^4	-	-	-	-	0.6×10^4
A_3	1.5×10^4	-	-	-	-	0.8×10^4
A_4	0.1×10^4	-	-	-	-	0.2×10^4
A_5	0.3×10^4	-	-	-	-	0.3×10^4
A_6	0.5×10^4	-	-	-	-	0.4×10^4
B_1	1.0×10^4	1.0×10^4	-	0.2×10^4	$0.2x10^4$	0.8×10^4
B_2	1.6×10^4	1.2×10^4	-	0.1×10^4	$0.1x\ 10^4$	1.4×10^4
B_3	2.0×10^4	1.5×10^4	-	-	0.2×10^4	2.0×10^4
B_4	1.0×10^4	1.0×10^4	-	-	-	0.2×10^4
B_5	1.4×10^4	0.6×10^4	-	-	-	0.5×10^4
B_6	1.8×10^4	0.5×10^4	-	-	-	0.8×10^4

Key
A_1 - A_3: 1st , 4th & 8th day Ambient at 28ºC for improved sample.
A_4 - A_6: 1st , 4th & 8th day Refrigerated at 4ºC for improved sample.
B_1 - B_3: 1st , 4th & 8th day Ambient at 28ºC for traditional sample.
B_4 - B_6: 1st , 4th & 8th day Refrigerated at 4ºC for traditional sample

Table 2. Microbes Isolated From Improved and Traditional Kunun–Zaki Beverage.

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Sample	Bacillus subtilis	Micrococcus Acidophilus	Streptococcus Lactis	Escherichia Coli	Lactobacillus plantarium	Pseudomonas aerogenosa	Saccharomyces cerevisiae	Rhizopus nigrican	Penicillium Sp
Ambient B	+	+	+	-	+	-	+	-	-
Refrigerated	+	+	+	-	-	-	+	-	-
Ambient B	+	+	+	+	+	+	+	+	+
Refrigerated	+	+	+	+	+	+	+	-	-

+ : Present
- : Absent

Table 3: Sensory Evaluation, Mean Score For Kunun – Zaki Beverage.

SAMPLE CODE	IMPROVED METHOD				TRADITIONAL METHOD			
	A_1	A_3	A_4	A_5	B_1	B_3	B_4	B_5
Colour	2.70 ± 0.10^b	1.80 ± 0.10^a	3.90 ± 0.30^e	3.20 ± 0.20^{cd}	1.93 ± 0.12^a	1.73 ± 0.21^a	3.30 ± 0.30^d	2.87 ± 0.35^{bc}
Taste	2.70 ± 0.10^b	1.80 ± 0.10^a	3.9 ± 0.35^e	3.20 ± 0.00^{cd}	1.93 ± 0.23^a	1.70 ± 0.10^a	3.30 ± 0.26^d	2.90 ± 0.30^{bc}
Consistency	2.70 ± 0.20^b	1.80 ± 0^a	3.90 ± 0.36^d	3.20 ± 0.34^c	1.93 ± 0.12^a	1.70 ± 0.00^a	3.30 ± 0.20^c	2.77 ± 0.15^b
Overall acceptability	4.03 ± 0.25^c	3.30 ± 0.26^b	3.20 ± 0.44^b	2.90 ± 0.17^b	1.70 ± 0.10^a	1.80 ± 0.30^a	1.93 ± 0.06^a	1.70 ± 0.30^a

Values with different superscript on the same are significantly different ($p<0.05$)

Key
A_1 & A_3: 1st & 8th day Ambient Improved sample.
A_4 & A_6: 1st & 8th day Refrigerated Improved sample.
B_1 & B_3: 1st & 8th day Ambient Traditional sample.
B_4 & B_6: 1st & 8th day Refrigerated Traditional sample.

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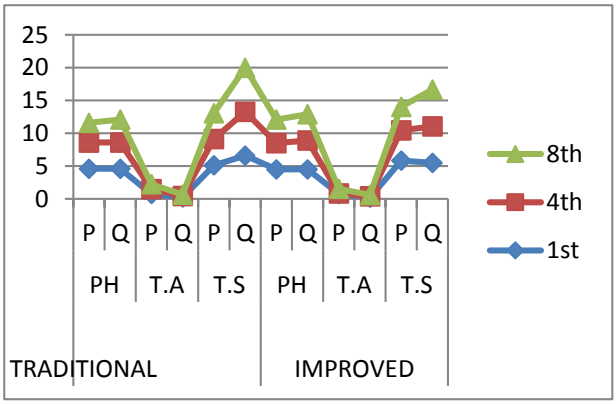

Figure2: Physicochemical analysis of kunun Zaki Beverages

KEY
pH
T.A = TITRABLE ACIDITY
T.S = TOTAL SOLUBLE
P= AMBIENT TEMPERATURE
Q= REFRIGERATION TEMPERATURE